L3 ANSWER 1 OF 1 MEDLINE
AN 97306204 MEDLINE
DN 97306204
TI Alkylation-induced apoptosis of embryonic stem cells in which the gene for
DNA-repair, methyltransferase, had been disrupted by gene targeting.
AU Tominaga Y; Tsuzuki T; Shiraishi A; Kawate H; Sekiguchi M
CS Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.
SO CARCINOGENESIS, (1997 May) 18 (5) 889-96.
Journal code: C9T. ISSN: 0143-3334.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199708

=> d l3 ab

L3 ANSWER 1 OF 1 MEDLINE
AB An enzyme O6-methylguanine-DNA methyltransferase (MGMT) catalyzes transfer
of a methyl group from O6-methylguanine and O4-methylthymine of alkylated DNA to its own molecule, thereby repairing the pre-mutagenic lesions in a single step reaction. Making use of gene targeting, we developed **mouse embryonic** stem (ES) **cell** lines deficient
in the methyltransferase. Quantitative immunoblot analysis and enzyme assay revealed that MGMT-/- cells, in which both alleles were disrupted, contained no methyltransferase protein while cells with one intact allele (MGMT+/-) contained about half the amount of protein carried by the parental MGMT+/+ cells. MGMT-/- cells have an extremely high degree of sensitivity to simple alkylating agents, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU), whereas MGMT+/- cells are slightly more sensitive to these agents, as compared with findings from normal cells. A high frequency of mutation was induced in MGMT-/- cells on exposure to a relatively low dose of MNNG.
Electrophoretic analyses of the DNAs as well as fluorochrome staining of the cells revealed that MGMT-/- cells treated with MNNG undergo apoptotic death, which occurs after G2-M arrest in the second cycle of cell proliferation.

L20 ANSWER 12 OF 26 MEDLINE
AN 97220036 MEDLINE
DN 97220036
TI Characterization of defective nucleotide excision repair in XPC mutant mice.
AU Cheo D L; Ruven H J; Meira L B; Hammer R E; Burns D K; Tappe N J; van Zeeland A A; Mullenders L H; Friedberg E C
CS Department of Pathology, The University of Texas Southwestern Medical Center, Dallas 75235, USA.
NC CA44247 (NCI)
SO MUTATION RESEARCH, (1997 Mar 4) 374 (1) 1-9.
Journal code: NNA. ISSN: 0027-5107.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199706
E

L30 ANSWER 2 OF 4 MEDLINE
AN 1998352147 MEDLINE
DN 98352147
TI Functional genomics in the post-genome era [published erratum appears in Mutat Res 1998 Dec 3;422(2):367].
AU Woychik R P; Klebig M L; Justice M J; **Magnuson T R**; Avner E D; Avrer ED/SS/[corrected to Avner ED]
CS Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44106, USA.. rpw@po.cwru.edu
SO MUTATION RESEARCH, (1998 May 25) 400 (1-2) 3-14. Ref: 47
Journal code: NNA. ISSN: 0027-5107.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Cancer Journals; Priority Journals
EM 199811

L30 ANSWER 2 OF 4 MEDLINE
AB As the biomedical research community enters the post-genome era, studying gene expression patterns and phenotypes in model organisms will be an important part of analyzing the role of genes in human health and disease.
New technologies involving DNA chips will improve the ability to evaluate the differential expression of a large number of genes simultaneously. Also, new approaches for generating mutations in mice will significantly decrease the cost and increase the rate of generating mutant lines that model human disease. Copyright 1998 Elsevier Science B.V. All rights reserved.

L7 ANSWER 1 OF 1 MEDLINE
AN 1998400236 MEDLINE
DN 98400236
TI Mammalian 3-methyladenine DNA glycosylase protects against the toxicity and clastogenicity of certain chemotherapeutic DNA cross-linking agents.
AU Allan J M; Engelward B P; Dreslin A J; Wyatt M D; Tomasz M; Samson L D
CS Department of Cancer Cell Biology, Harvard School of Public Health, Boston, Massachusetts 02115, USA.
NC RO1 CA55042 (NCI)
RO1CA28681 (NCI)
CA73135-01 (NCI)
+
SO CANCER RESEARCH, (1998 Sep 1) 58 (17) 3965-73.
Journal code: CNF. ISSN: 0008-5472.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199811
EW 19981103

=> D L7 AB

L7 ANSWER 1 OF 1 MEDLINE
AB DNA repair status is recognized as an important determinant of the clinical efficacy of cancer chemotherapy. To assess the role that a mammalian DNA glycosylase plays in modulating the toxicity and clastogenicity of the chemotherapeutic DNA cross-linking alkylating agents, we compared the sensitivity of wild-type murine cells to that of isogenic cells bearing homozygous null mutations in the 3-methyladenine DNA glycosylase gene (Aag). We show that Aag protects against the toxic and clastogenic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea and mitomycin C (MMC), as measured by cell killing, sister chromatid exchange, and chromosome aberrations. This protection is accompanied by suppression of apoptosis and a slightly reduced p53 response. Our results identify 3-methyladenine DNA glycosylase-initiated base excision repair as a potentially important determinant of the clinical efficacy and, possibly, the carcinogenicity of these widely used chemotherapeutic agents. However, Aag does not contribute significantly to protection against the toxic and clastogenic effects of several chemotherapeutic nitrogen mustards (namely, mechlorethamine, melphalan, and **chlorambucil**), at least in the **mouse embryonic** stem **cells** used here. We also compare the Aag null phenotype with the Fanconi anemia phenotype, a human disorder characterized by cellular hypersensitivity to DNA cross-linking agents, including MMC. Although Aag null cells are sensitive to MMC-induced growth delay and cell cycle arrest, their sensitivity is modest compared to that of Fanconi anemia cells.

L8 ANSWER 2 OF 2 MEDLINE
AN 97415542 MEDLINE
DN 97415542
TI Enhanced apoptosis in metallothionein null cells.
AU Kondo Y; Rusnak J M; Hoyt D G; Settineri C E; Pitt B R; Lazo J S
CS Department of Pharmacology, University of Pittsburgh, Pennsylvania 15261, USA.
NC CA61299 (NCI)
DK46935 (NIDDK)
HL32154 (NHLBI)
SO MOLECULAR PHARMACOLOGY, (1997 Aug) 52 (2) 195-201.
Journal code: NGR. ISSN: 0026-895X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199711

=> D
L8 2 AB

L8 ANSWER 2 OF 2 MEDLINE
AB Metallothioneins (MTs) are major intracellular, zinc-binding proteins with antioxidant properties. **Mouse embryonic cells** null for MT due to loss of functional MT I and II genes (MT-/-) were more susceptible to apoptotic death after exposure to tert-butyl hydroperoxide or the anti-cancer agents cytosine arabinoside, bleomycin, **melphalan**, and cis-dichlorodiammineplatinum(II) compared with wild-type **mouse embryonic cells** (MT+/+). We measured basal levels of the tumor suppressor protein p53 and the death effector protein Bax and found the basal levels of both proteins were higher in MT null cells compared with MT+/+ cells. After treatment with the DNA-damaging agent cis-dichlorodiammineplatinum(II), p53 protein levels were induced in both MT+/+ and MT-/- cells with MT null cells always maintaining the highest p53 levels. The elevated sensitivity to apoptosis was not restricted to embryonic cells. Primary pulmonary fibroblasts were isolated from distinct litters of MT null, heterozygous, and wild-type mice, and all had undetectable basal MT levels. Zinc exposure increased MT levels in the wild-type and heterozygous fibroblasts but not in the MT null fibroblasts. Consistent with the induced MT levels, we found MT+/+ and MT+/- embryonic cells were less sensitive to cis-dichlorodiammineplatinum(II)-induced apoptosis compared with MT-/- cells. Our results implicate MT as a stress-responsive factor that can regulate apoptotic engagement.

L14 ANSWER 2 OF 2 MEDLINE
AN 95408305 MEDLINE
DN 95408305
TI Gene targeting of DT-diaphorase in **mouse embryonic** stem **cells**: establishment of null mutant and its **mitomycin C**-resistance.
AU Yoshida T; Tsuda H
CS Life Science Research Laboratory, Japan Tobacco Inc., Kanagawa..
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1995 Sep 14) 214 (2)
701-8.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199512

=> D
L14 2 AB

L14 ANSWER 2 OF 2 MEDLINE
AB It is generally accepted that DT-diaphorase is primarily involved in the detoxification of quinone compounds and is capable of metabolically activating some cancer chemotherapeutic quinones including **mitomycin C**. However, these conclusions have mainly been drawn from the experiments using the DT-diaphorase inhibitor, dicoumarol. To understand directly the roles of this enzyme in quinone metabolism, we have established heterozygously and homozygously DT-diaphorase-targeted mutant embryonic stem (ES) cells by homologous recombination. Cytotoxicity experiments using these cells clearly demonstrated that DT-diaphorase acts as an activator of **mitomycin C** in ES cells. These mutant cell lines seem to be very useful for investigating the functions of DT-diaphorase including the bioactivation and detoxification of quinone species. The generation of a DT-diaphorase-targeted mouse is under investigation.

L15 ANSWER 1 OF 2 MEDLINE
AN 1998408012 MEDLINE
DN 98408012
TI Protein synthesis and transcriptional inhibitors control **N-methyl-N'-nitro-N-nitrosoguanidine**-induced levels of APC mRNA in a p53-dependent manner.
AU Jaiswal A S; Narayan S
CS Sealy Center for Oncology and Hematology, University of Texas Medical Branch, Galveston, TX 77555-1048, USA.
SO INTERNATIONAL JOURNAL OF ONCOLOGY, (1998 Oct) 13 (4) 733-40. Journal code: CX5. ISSN: 1019-6439.
CY Greece
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199902
EW 19990204

L15 ANSWER 1 OF 2 MEDLINE
AB In the present study, we show that treatment of wild-type (p53+/+) **mouse embryonic** fibroblast (MEF) **cells** with a DNA-alkylating agent, N-methyl-N'-nitro-N-nitro-soguanidine (MNNG), resulted in increased levels of adenomatous polyposis coli (APC) mRNA compared to p53 gene-knocked out (p53-/-) MEF cells, indicating that p53 is required for APC expression after alkylation damage. By using HCT-116 colon cancer cells (containing wild-type p53 gene) or p53-/- MEF cells transfected with a pCMV-p53 overexpression plasmid [p53-/-(CMV-p53)], we show that p53 is a labile factor for APC gene expression, and that pretreating HCT-116 cells with a protein synthesis inhibitor, cycloheximide (CHX), inhibited MNNG-induced APC mRNA levels by inhibiting p53 protein synthesis. The effect of CHX on p53 protein synthesis was reversible, as the withdrawal of CHX permitted p53 protein synthesis to resume with a concomitant increase in APC mRNA levels after MNNG treatment. To examine whether p53 regulates APC gene expression at the transcriptional level, we treated HCT-116 or p53-/-(CMV-p53) MEF cells with 5,6-dichloro-1-beta-D-ribofuranosylbenzamidazole (DRB; a transcriptional inhibitor), before the MNNG treatment. Although treatment of cells with DRB resulted in increased p53 protein levels, that the APC mRNA levels were decreased suggests that p53 may enhance APC gene expression upstream of the transcriptional machinery where DRB interacts. That the withdrawal of DRB, and subsequent MNNG treatment, increased the level of APC mRNA indicated that the binding of DRB to the transcriptional machinery was reversible.

L15 ANSWER 2 OF 2 MEDLINE
AN 97306204 MEDLINE
DN 97306204
TI Alkylation-induced apoptosis of embryonic stem cells in which the gene for
DNA-repair, methyltransferase, had been disrupted by gene targeting.
AU Tominaga Y; Tsuzuki T; Shiraishi A; Kawate H; Sekiguchi M
CS Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.
SO CARCINOGENESIS, (1997 May) 18 (5) 889-96.
Journal code: C9T. ISSN: 0143-3334.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199708

L15 ANSWER 2 OF 2 MEDLINE
AB An enzyme O6-methylguanine-DNA methyltransferase (MGMT) catalyzes transfer
of a methyl group from O6-methylguanine and O4-methylthymine of alkylated DNA to its own molecule, thereby repairing the pre-mutagenic lesions in a single step reaction. Making use of gene targeting, we developed **mouse embryonic** stem (ES) **cell** lines deficient
in the methyltransferase. Quantitative immunoblot analysis and enzyme assay revealed that MGMT-/- cells, in which both alleles were disrupted, contained no methyltransferase protein while cells with one intact allele (MGMT+/-) contained about half the amount of protein carried by the parental MGMT+/+ cells. MGMT-/- cells have an extremely high degree of sensitivity to simple alkylating agents, **N-methyl-N'-nitro-N-nitrosoguanidine** (MNNG)
and N-methyl-N-nitrosourea (MNU), whereas MGMT+/- cells are slightly more sensitive to these agents, as compared with findings from normal cells. A high frequency of mutation was induced in MGMT-/- cells on exposure to a relatively low dose of MNNG. Electrophoretic analyses of the DNAs as well as fluorochrome staining of the cells revealed that MGMT-/- cells treated with MNNG undergo apoptotic death, which occurs after G2-M arrest in the second cycle of cell proliferation.

L19 ANSWER 1 OF 2 MEDLINE
AN 97220036 MEDLINE
DN 97220036
TI Characterization of defective nucleotide excision repair in XPC mutant mice.
AU Cheo D L; Ruven H J; Meira L B; Hammer R E; Burns D K; Tappe N J; van Zeeland A A; Mullenders L H; Friedberg E C
CS Department of Pathology, The University of Texas Southwestern Medical Center, Dallas 75235, USA.
NC CA44247 (NCI)
SO MUTATION RESEARCH, (1997 Mar 4) 374 (1) 1-9.
Journal code: NNA. ISSN: 0027-5107.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199706
E

L19 ANSWER 1 OF 2 MEDLINE
AB Nucleotide excision repair (NER) is a fundamental process required for maintaining the integrity of the genome in cells exposed to environmental DNA damage. Humans defective in NER suffer from the hereditary cancer-prone disease xeroderma pigmentosum. In order to model this disease in mice a mutation in the mouse XPC gene was generated and used to replace a wild-type XPC allele in **mouse embryonic** stem **cells** by homologous recombination. These cells were used to derive XPC mutant mice. Fibroblasts from mutant embryos were more sensitive to the cytotoxic effects of **ultraviolet light** than wild-type and heterozygous cells. Repair synthesis of DNA following irradiation with **ultraviolet light** was reduced in these cells, indicating a defect in NER. Additionally, XPC mutant embryo fibroblasts were specifically defective in the removal of pyrimidine (6-4) pyrimidone photoproducts from the non-transcribed strand of the transcriptionally active p53 gene. Mice defective in the XPC gene appear to be an excellent model for studying the role of NER and its interaction with other proteins in the molecular pathogenesis of cancer in mammals following exposure to environmental carcinogens.

L19 ANSWER 2 OF 2 MEDLINE
AN 97194977 MEDLINE
DN 97194977
TI Quantification of XPA gene expression levels in human and mouse cell lines
by competitive RT-PCR.
AU Layher S K; Cleaver J E
CS Laboratory of Radiobiology and Environmental Health, University of California, San Francisco 94143-0750, USA.
NC 5 T32 ES07106 (NIEHS)
SO MUTATION RESEARCH, (1997 Jan 31) 383 (1) 9-19.
Journal code: NNA. ISSN: 0027-5107.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199705
EW 19970504

L19 ANSWER 2 OF 2 MEDLINE
AB Expression of the mammalian photoproduct-specific DNA-binding protein XPA has been quantified by competitive reverse transcription-polymerase chain reaction, a method that provides relative numbers of mRNA molecules per cell. Human primary and simian virus (SV)40-transformed fibroblasts had 4.8 and 8.4 transcripts per **cell**, respectively; **mouse embryonic** and SV40-transformed fibroblasts had 6.7 and 5.5 transcripts per cell, respectively. None of these differences are significant, and the mean value of 5 to 8 transcripts per cell indicates that XPA is expressed as a low-abundance mRNA. Two cell lines transfected with XPA on a conditional promoter showed different numbers of XPA mRNA molecules, consistent with their respective responses to an inducer and their sensitivity to **ultraviolet light**. The similarity of results in human and mouse cells shows that a difference in XPA expression cannot account for the greater repair of nontranscribed DNA in human cells.

L20 ANSWER 10 OF 26 MEDLINE
AN 1998115883 MEDLINE
DN 98115883
TI **X-ray**-induced mutations in **mouse** **embryonic** stem **cells**.
AU Thomas J W; LaMantia C; Magnuson T
CS Department of Genetics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4955, USA.
NC HD26722 (NICHD)
NS32779 (NINDS)
HD07104 (NICHD)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Feb 3) 95 (3) 1114-9.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199805
E

L20 ANSWER 10 OF 26 MEDLINE
AB Deletion complexes consisting of multiple chromosomal deletions induced at
single loci can provide a means for functional analysis of regions spanning several centimorgans in model genetic systems. A strategy to identify and map deletions at any cloned locus in the mouse is described here. First, a highly polymorphic, germ-line competent F1(129/Sv-+Tyr+p x CAST/Ei) **mouse embryonic** stem **cell** line was established. Then, **x-ray** and UV-induced mutagenesis was performed to determine the feasibility of generating deletion complexes throughout the mouse genome. Reported here are the selection protocols, induced mutation frequencies, cytogenetic and extensive molecular analysis of mutations at the X-chromosome-linked hypoxanthine phosphoribosyltransferase (Hprt) locus and at the neural cell adhesion molecule (Ncam) locus located on chromosome 9. Mutation analysis with PCR-based polymorphic microsatellite markers revealed deletions of <3 cM at the Hprt locus, whereas results consistent with deletions covering >28 cM were observed at the Ncam locus. Fluorescence in situ hybridization with a chromosome 9 paint revealed that some of the Ncam deletions were accompanied by complex chromosome rearrangements. In addition, deletion mapping in combination with loss of heterozygosity of microsatellite markers revealed a putative haploinsufficient region distal to Ncam. These
data indicate that it is feasible to generate **x-ray** -induced deletion complexes in **mouse embryonic** stem **cells**.

L20 ANSWER 9 OF 26 MEDLINE
AN 1998149969 MEDLINE
DN 98149969
TI Generation of **radiation**-induced deletion complexes in the mouse genome using embryonic stem cells.
AU You Y; Browning V L; Schimenti J C
CS The Jackson Laboratory, Bar Harbor, Maine 04609, USA.
SO METHODS, (1997 Dec) 13 (4) 409-21. Ref: 42
Journal code: CPO. ISSN: 1046-2023.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199805
EW 19980503

L20 ANSWER 9 OF 26 MEDLINE
AB As the genetic and physical mapping stage of the Human Genome Project nears completion, the focus is shifting toward the development of technologies for high-throughput analysis of gene function. Whereas DNA sequencing will enable the assignment of presumed function to a large number of genes in mice and humans, it is clear that the great majority of genes will have to be evaluated in vivo to accurately assess their role in a complex organism. While gene targeting in **mouse** **embryonic** stem (ES) **cells** is the current method of choice for the characterization of gene function in mice, it remains relatively labor intensive and lacks the throughput required for analysis of genome function on a large scale. Alternative methods of efficient mutagenesis will clearly be required for this task. Chromosomal deletions are powerful tools in the genetic analysis of complex genomes, enabling the systematic identification and localization of functional units along defined chromosomal regions. Not only are deletions useful for the identification of genetic functions, but they serve as mapping reagents for existing mutations or traits. While their use has been an essential tool in Drosophila genetics, classical mutagenesis in mice has been logistically impractical for generating deletions. We have previously described an efficient method for generating **radiation**-induced deletion complexes at defined regions in the genome using ES cells. In this article, we detail the methodological aspects of this technology and describe the applications of chromosomal deletions for characterizing gene function in ways that make optimal use of the information generated by the first stage of the Genome Project. Copyright 1997 Academic Press.

L20 ANSWER 7 OF 26 MEDLINE
AN 1998369178 MEDLINE
DN 98369178
TI BRCA1 required for transcription-coupled repair of oxidative DNA damage.
AU Gowen L C; Avrutskaya A V; Latour A M; Koller B H; Leadon S A
CS Curriculum in Genetics and Molecular Biology and Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
NC CA70490 (NCI)
IP50CA58223 (NCI)
CA40453 (NCI)
SO SCIENCE, (1998 Aug 14) 281 (5379) 1009-12.
Journal code: UJ7. ISSN: 0036-8075.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199810
EW 19981005

L20 ANSWER 7 OF 26 MEDLINE
AB The breast and ovarian cancer susceptibility gene BRCA1 encodes a zinc finger protein of unknown function. Association of the BRCA1 protein with the DNA repair protein Rad51 and changes in the phosphorylation and cellular localization of the protein after exposure to DNA-damaging agents are consistent with a role for BRCA1 in DNA repair. Here, it is shown that **mouse embryonic** stem **cells** deficient in BRCA1 are defective in the ability to carry out transcription-coupled repair of oxidative DNA damage, and are hypersensitive to ionizing **radiation** and hydrogen peroxide. These results suggest that BRCA1 participates, directly or indirectly, in transcription-coupled repair of oxidative DNA damage.

L21 ANSWER 2 OF 7 MEDLINE
AN 1998147784 MEDLINE
DN 98147784
TI I-SceI-induced gene replacement at a natural locus in embryonic stem cells.
AU Cohen-Tannoudji M; Robine S; Choulika A; Pinto D; El Marjou F; Babinet C; Louvard D; Jaisser F
CS Unite de Biologie du Developpement, CNRS URA 1960, Institut Pasteur, Paris, France.
SO MOLECULAR AND CELLULAR BIOLOGY, (1998 Mar) 18 (3) 1444-8.
Journal code: NGY. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199805
EW 19980503

L21 ANSWER 2 OF 7 MEDLINE
AB Gene targeting is a very powerful tool for studying mammalian development and physiology and for creating models of human diseases. In many instances, however, it is desirable to study different modifications of a target gene, but this is limited by the generally low frequency of homologous recombination in mammalian cells. We have developed a novel gene-targeting strategy in **mouse embryonic** stem **cells** that is based on the induction of endogenous gap repair processes at a defined location within the genome by induction of a double-**strand break** (DSB) in the gene to be mutated. This strategy was used to knock in an NH2-ezrin mutant in the villin gene, which encodes an actin-binding protein expressed in the brush border of the intestine and the kidney. To induce the DSB, an I-SceI yeast meganuclease restriction site was first introduced by gene targeting to the villin gene, followed by transient expression of I-SceI. The repair of the ensuing DSB was achieved with high efficiency (6 x 10[-6]) by a repair shuttle vector sharing only a 2.8-kb region of homology with the villin gene and no negative selection marker. Compared to conventional gene-targeting experiments at the villin locus, this represents a 100-fold stimulation of gene-targeting frequency, notwithstanding a much lower length of homology. This strategy will be very helpful in facilitating the targeted introduction of several types of mutations within a gene of interest.

6 ANSWER 20 OF 20 MEDLINE
AN   86040459   MEDLINE
DN   86040459
TI   An inherited limb deformity created by insertional mutagenesis in a
     transgenic mouse.
AU   **Woychik R P**; Stewart T A; Davis L G; D'Eustachio P; Leder P
SO   NATURE, (1985 Nov 7-13) 318 (6041) 36-40.
     Journal code: NSC. ISSN: 0028-0836.
CY   ENGLAND: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals; Cancer Journals
EM   198602

=> D
L26 20 AB

L26 ANSWER 20 OF 20 MEDLINE
AB   We have created an insertional **mutation** that leads to a severe
     defect in the pattern of limb formation in the developing mouse. The
novel
     recessive **mutation** is phenotypically identical and
     non-complementary to two previously encountered limb deformity mutations,
     and is closely linked to a dominant **mutation** that gives rise to
     a related limb dysmorphism. The inserted element thus provides a
molecular
     genetic link with the control of pattern formation in the mammalian
     embryo.

L26 ANSWER 19 OF 20 MEDLINE
AN 90207241 MEDLINE
DN 90207241
TI Molecular and genetic characterization of a radiation-induced structural rearrangement in mouse chromosome 2 causing mutations at the limb deformity and agouti loci.
AU **Woychik R P**; Generoso W M; Russell L B; Cain K T; Cacheiro N L; Bultman S J; Selby P B; Dickinson M E; Hogan B L; Rutledge J C
CS Biology Division, Oak Ridge National Laboratory, TN..
NC IAG 222Y01-ES-10067 (NIEHS)
RO1 EY08000-01 (NEI)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1990 Apr) 87 (7) 2588-92.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199007

L26 ANSWER 19 OF 20 MEDLINE
AB Molecular characterization of mutations in the mouse, particularly those involving agent-induced major structural alterations, is proving to be useful for correlating the structure and expression of individual genes with their function in the whole organism. Here we present the characterization of a radiation-induced **mutation** that simultaneously generated distinct alleles of both the limb deformity (ld) and agouti (a) loci, two developmentally important regions of chromosome 2
normally separated by 20 centimorgans. Cytogenetic analysis revealed that an interstitial segment of chromosome 17 (17B- 17C; or, possibly, 17A2-17B) had been translocated into the distal end of chromosome 2, resulting in a smaller-than-normal chromosome 17 (designated 17del) and a larger form of chromosome 2 (designated 2(17). Additionally, a large interstitial segment of the 2(17) chromosome, immediately adjacent and proximal to the insertion site, did not match bands 2E4-2H1 at corresponding positions on a normal chromosome 2. Molecular analysis detected a DNA rearrangement in which a portion of the ld locus was joined
to sequences normally tightly linked to the a locus. This result, along with the genetic and cytogenetic data, suggests that the alleles of ld and
a in this radiation-induced **mutation**, designated ldIn2 and ajIn2, were associated with DNA breaks caused by an inversion of an interstitial segment in the 2(17) chromosome.

199304

L26 ANSWER 15 OF 20 MEDLINE
AN 91376087 MEDLINE
DN 91376087
TI Molecular characterization of a region of DNA associated with mutations at
the agouti locus in the mouse.
AU Bultman S J; Russell L B; Gutierrez-Espeleta G A; **Woychik R P**
CS Biology Division, Oak Ridge National Laboratory, TN..
NC IAG 22Y01-ES-10067 (NIEHS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1991 Sep 15) 88 (18) 8062-6.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
E

L26 ANSWER 15 OF 20 MEDLINE
AB Molecular characterization of a radiation-induced agouti (a)-locus **mutation** has resulted in the isolation of a segment of DNA that maps at or near the a locus on chromosome 2 in the mouse. This region of DNA is deleted in several radiation- or chemical-induced homozygous-lethal a-locus mutations and is associated with specific DNA structural alterations in two viable a-locus mutations. We propose that DNA probes from this region of chromosome 2 will be useful for ultimately characterizing the individual gene or genes associated with a-locus function.

L26 ANSWER 4 OF 20 MEDLINE
AN 95402699 MEDLINE
DN 95402699
TI Molecular and phenotypic characterization of a new mouse insertional **mutation** that causes a defect in the distal vertebrae of the spine.
AU Schrick J J; Dickinson M E; Hogan B L; Selby P B; **Woychik R P**
CS University of Tennessee, Graduate School for Biomedical Sciences, Oak Ridge 37831-8080, USA.
NC RO1 HD-25323 (NICHD)
SO GENETICS, (1995 Jul) 140 (3) 1061-7.
Journal code: FNH. ISSN: 0016-6731.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
E

L26 ANSWER 4 OF 20 MEDLINE
AB We have identified and characterized the phenotype of a new insertional **mutation** in one line of transgenic mice. Mice carrying this **mutation**, which we have designated TgN(Imusd)370Rpw, display undulations of the vertebrae giving rise to a novel kinky-tail phenotype. Molecular characterization of the insertion site indicates that the transgene integration has occurred without any substantial alterations in the structure of the host sequences. Using probes that flank the insertion site, we have mapped the **mutation** to chromosome 5 near the semidominant **mutation**, thick tail (Tht). Thick tail does not complement the TgN(Imusd)370Rpw **mutation**; compound mutants containing one copy of each **mutation** display a more severe phenotype than either **mutation** individually.

L26 ANSWER 3 OF 20 MEDLINE
AN 96187435 MEDLINE
DN 96187435
TI Insertional mutagenesis and molecular analysis of a new gene associated with polycystic kidney disease.
AU Yoder B K; Richards W G; Sweeney W E; Wilkinson J E; Avener E D; **Woychik R P**
CS Biology Division, Oak Ridge National Laboratory, TN 37831-8080, USA.
NC 1 RO1 DK45633-01 (NIDDK)
5 RO1 DK44875 (NIDDK)
SO PROCEEDINGS OF THE ASSOCIATION OF AMERICAN PHYSICIANS, (1995 Oct) 107 (3) 314-23.
Journal code: CDQ. ISSN: 1081-650X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
E

L26 ANSWER 3 OF 20 MEDLINE
AB We have identified a new insertional **mutation** in the mouse (TgN737Rpw) that causes a phenotype that closely resembles human autosomal recessive polycystic kidney disease. The renal pathology in these mutants first presents itself as a dilation of the proximal tubules, which is quickly followed by the development of cystic lesions in the collecting ducts. The livers in the mutant animals develop a variable lesion depending upon the genetic background. We have cloned the mutant locus and have isolated and characterized a gene, Tg737, whose expression is disrupted in the mutant animals. Expression of the Tg737 gene can normally be detected using the Northern blot analysis at low levels in a variety of tissues, including the kidney and liver. Using the in situ hybridization procedure, expression of the Tg737 mRNA can be detected in the collecting ducts of adult kidneys and in portions of the embryonic day 15.5 kidney. Most important, we have corrected the defective kidney trait by expressing the wild-type cDNA as a transgene in the mutant animals. The human homologue of the Tg737 gene has also been cloned and mapped to human chromosome 13.

L32 ANSWER 6 OF 10 MEDLINE
AN 97042002 MEDLINE
DN 97042002
TI Evidence that two phenotypically distinct mouse PKD **mutations**, bpk and jcpk, are allelic.
AU Guay-Woodford L M; Bryda E C; Christine B; Lindsey J R; Collier W R; **Avner E D**; D'Eustachio P; Flaherty L
CS Department of Medicine, University of Alabama at Birmingham, USA.
NC R01DK51034-01 (NIDDK)
DK45616 (NIDDK)
GM50283 (NIGMS)
SO KIDNEY INTERNATIONAL, (1996 Oct) 50 (4) 1158-65.
Journal code: KVB. ISSN: 0085-2538.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199705
E

L32 ANSWER 6 OF 10 MEDLINE
AB Numerous mouse models of polycystic kidney disease (PKD) have been described. All of these diseases are transmitted as single recessive traits and in most, the phenotypic severity is influenced by the genetic background. However, based on their genetic map positions, none of these loci appears to be allelic and none are candidate modifier loci for any other mouse PKD **mutation**. Previously, we have described the mouse bpk **mutation**, a model that closely resembles human autosomal recessive polycystic kidney disease. We now report that the bpk **mutation** maps to a 1.6 CM interval on mouse Chromosome 10, and that the renal cystic disease severity in our intersubspecific intercross progeny is influenced by the genetic background. Interestingly, bpk co-localizes with jcpk, a phenotypically-distinct PKD **mutation**, and complementation testing indicates that the bpk and jcpk **mutations** are allelic. These data imply that distinct PKD phenotypes can result from different **mutations** within a single gene. In addition, based on its map position, the bpk locus is a candidate genetic modifier for jck, a third phenotypically-distinct PKD **mutation**.

L39 ANSWER 2 OF 4 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
AN 1996:298654 CAPLUS
DN 124:335031
TI The stem-cell test: an in vitro assay for teratogenic potential. Results of a blind trial with 25 compounds
AU Newall, D. R.; Beedles, K. E.
CS Genetic and Reproductive Toxicology, Glaxo Wellcome Research and Development, Ware, Herts, SG12 0DP, UK
SO Toxicol. in Vitro (1996), 10(2), 229-240
CODEN: TIVIEQ; ISSN: 0887-2333
DT Journal
LA

L39 ANSWER 3 OF 4 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2
AB The stem-cell test is a novel assay for teratogenic potential which uses a propagated cell line. **Mouse embryonic** stem **cells** (ESC) are maintained in an undifferentiated state in the presence of leukemia inhibitory factor (LIF). On removing LIF, the cells differentiate into an embryonic endoderm that is morphol. distinct from ESC. Colonies of ESC are maintained from which cells can be harvested daily, and these cells, when washed free of LIF, form a population of differentiating cells on which the effects of chems. can be tested. The conditions under which differentiating ESC can be substituted for rat primary embryonic cells in a micromass test protocol have been detd., and the effects of 25 compds. investigated in a blind trial. The stem-cell test predicted the teratogenicity of these compds. with a similar sensitivity and specificity to the micromass test, with the advantage that the test uses a propagated cell line; there is no use of animals.

L39 ANSWER 3 OF 4 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2
AN 1994:648154 CAPLUS
DN 121:248154
TI The stem-cell test - a novel in vitro assay for teratogenic potential
AU Newall, D. R.; Beedles, K. E.
CS Genetic and Reproductive Toxicology, Glaxo Group Research Ltd., Herts, SG12 ODP, UK
SO Toxicol. in Vitro (1994), 8(4), 697-701
CODEN: TIVIEQ; ISSN: 0887-2333
DT Journal
LA English

L39 ANSWER 3 OF 4 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2
AB The stem-cell test is a novel assay for teratogenic potential which uses a propagated cell line. **Mouse embryonic** stem **cells** (ESC) are maintained in an undifferentiated state in the presence of leukemia inhibitory factor (LIF). On removing LIF, the cells differentiate into an embryonic endoderm that is morphol. distinct from ESC. Colonies of ESC are maintained from which cells can be harvested daily, and these cells, when washed free of LIF, form a population of differentiating cells on which the effects of chems. can be tested. The conditions under which differentiating ESC can be substituted for rat primary embryonic cells in a micromass test protocol have been detd., and the effects of 25 compds. investigated in a blind trial. The stem-cell test predicted the teratogenicity of these compds. with a similar sensitivity and specificity to the micromass test, with the advantage that the test uses a propagated cell line; there is no use of animals.